Growth of *Clostridium sporogenes* and *Staphylococcus aureus* at Different Temperatures in Cooked Corned Beef Made with Reduced Levels of Sodium Chloride

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ABSTRACT

Cooked corned beef made with normal (ca. 2.5%) or a reduced (ca. 1.5%) level of salt were inoculated with either clostridial spores or with staphylococci and incubated at temperatures ranging from 5 - 30°C. Growth of indigenous microflora, staphylococci, or clostridia was similar at both salt levels at a given incubation temperature. However, increasing the abuse temperature greatly increased the growth of all organisms. Outgrowth of clostridial spores occurred in ground cooked corned beef which contained the normal residual nitrite of 40 - 45 ppm; readdition of nitrite to 150 ppm at the time of inoculation markedly reduced growth. Gas production was not a good indicator of clostridial growth.

INTRODUCTION

REDUCING DIETARY SODIUM has been recommended to decrease the incidence of hypertension and subsequent occurrences of cardiovascular disease, stroke, renal failure, and decreased life span in individuals susceptible to these conditions (Pearson and Wolzak, 1982; Sebranek et al., 1983). Processed meat products are an important source of dietary sodium. For example, corned beef loaf contains an average of 1,037 mg sodium/100g (USDA, 1980). However, the sodium content within a class of meat products currently in the marketplace often ranges more than two-fold, e.g., the content in hams ranged from 654 - 2004 mg sodium/100g (Sebranek et al., 1983).

Sodium chloride has three functions in meat products: providing and enhancing flavor, solubilizing proteins to create desired texture, and controlling microbial growth to enhance shelf life and inhibit pathogens (Ingram and Kitchell, 1967; Terrell, 1983). The microbiological preservation and safety of most meat products is a result of a combination of salt and nitrite levels, pH, heat processing, vacuum packaging, and refrigeration. Few studies have described the changes in microbiological growth in semi-preserved meat products with reductions of 20 - 50% from current salt levels (Rieman et al., 1972; Smith et al., 1983). Terrell and Brown (1981) found that only a relatively high salt level (brine content above 4.5%) reduced the growth of aerobic bacteria in vacuum packaged frankfurters. Sofos (1983) reduced the salt content of frankfurters from 2.5% to 1.5% and observed that pH of the batter was more important than salt level in determining growth of psychrotropic and mesophilic bacteria. Kraft (1983) found that a similar salt reduction in bologna, bacon, or ham did not adversely affect shelf life. Greenberg et al. (1959) using a model ham product showed that *Clostridium botulinum* growth and toxin production were not inhibited at less than 9% brine. Toxin was formed when brine contents exceeded 6.25% even though the ham lacked signs of obvious spoilage. Whiting et al. (1984) found temperature to be a more important factor than salt in controlling growth of aerobic bacteria, facultative bacteria, and *Staphylococcus aureus* in frankfurters. *Clostridium sporogenes* spore outgrowth was effectively inhibited, probably by the nitrite.

This study will determine the growth of bacteria in cooked and vacuum packaged corned beef made with a reduced level of sodium chloride. Growth of the organisms will be followed at various abuse temperatures.

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MATERIALS & METHODS

Corned beef

Beef bottom round was obtained fresh from a local distributor. The meat was trimmed of excess surface fat and connective tissue and portions from the same postmortem muscles were distributed to the various curing treatments to minimize pH and compositional differences that were not a consequence of the curing itself. The manufacture of cooked corned beef followed the procedures given by Komarick et al. (1974). The curing pickle contained 0.16% $NaNO_3$, 0.16% $NaNO_2$, 0.44% sodium ascorbate, 2.4% sucrose, and 2.7% sodium tripolyphosphate. The sodium chloride content of the curing pickle was varied so that adding the pickle at 15% of the green weight would yield corned beef with the desired salt content. Approximately two-thirds of the pickle was injected into the meat with a single needle pumping system, and the remainder was added as a cover pickle. Bags containing a 3 - 6 kg piece of round plus pickle were vacuum sealed to have continuous contact of the meat and pickle and were cured at 6°C for 5 days.

After curing, the corned beef was cooked in the bag with the small amount of remaining brine by immersion in 80 - 90°C water until temperature probes inserted in the center of the piece indicated 71°C. The cooked corned beef was rapidly cooled in ice water, cut into approximately 1kg pieces, and frozen at −18°C until needed. Enough corned beef was made so that replicate runs of an experiment could be made from one batch to minimize variation in salt content.

Pieces of cooked corned beef were thawed at 1°C and sliced with a meat slicer. Twenty-five grams of slices were placed into vacuum pouches. They were then inoculated, vacuum sealed (0.97 bar), and stored at various temperatures.

Because of the difficulty in achieving an exact salt level after curing and cooking, portions of the cooked corned beef containing an average of 1.2% salt were ground uniformly, and additional salt and/or a sodium nitrite solution was added to achieve up to 4.0% salt and 150 ppm $NaNO_2$. After mixing and standing for several hours, 25g were weighed into pouches, inoculated with clostridia, vacuum sealed, heat shocked at 80°C for 10 min, and stored.

Chemical analyses

Water content of the cooked corned beef was determined by air drying procedures (AOAC, 1980). Sodium content was obtained by dry ashing at 525°C, dissolving in nitric acid, and measuring by atomic absorption spectroscopy (AOAC, 1980). Residual nitrite was estimated by the Griess reagent following the AOAC (1980) procedure except that sulfanilic acid and 1-naphthalyamine were added simultaneously.

Microbiological methods

The microbiological techniques were similar to those used in an equivalent study of frankfurters (Whiting et al., 1984). Aerobic plate counts were made by homogenizing the entire contents of a pouch with 50 mL of 0.1% peptone water in a Stomacher 400 for 2 min. Three milliliters were taken and dilutions were made with peptone water; and 0.1 mL aliquot was spread on APT agar (Difco) and incubated at 20°C.

A *S. aureus* 196E stock culture was grown with shaking in 250 mL Brain Heart Infusion (BHI) (Difco) at 37°C for 48 hr. Aliquots of 1 or 10 mL were placed into vials, frozen in dry-ice acetone, and stored at −13°C. Thawed samples plated on TSA agar (Difco) containing 7.0% additional salt (TSAS agar) had a viable count of 7×10^7 CFU/mL. Added salt inhibits most bacterial species, but this strain of *S. aureus* grows consistently as golden-colored colonies. To inoculate the corned beef, an aliquot of *S. aureus* stock culture was thawed and diluted so that 0.25 mL inoculated into the pouches would contain approximately 10^3 organisms/g.

After a predetermined storage time, the entire contents of a pouch were transferred to a Stomacher bag. Fifty milliliters of peptone water were added, part of the peptone water was used to rinse the pouch. Three milliliters were removed for diluting and plating, and the *S. aureus* were enumerated as golden colonies on TSAS agar after incubation at 37°C for 48 hr.

C. sporogenes (B1219) is a nontoxigenic anaerobic spore former similar go the proteolytic strains of *C. botulinum*. A spore suspension was prepared in beef heart infusion according to Santo-Goldoni et al. (1980). Spores were inoculated onto the cooked corned beef at 9.4×10^3 spores/g. The pouches were vacuum sealed, heat shocked at 80°C for 10 min, and stored. After the desired storage, 50 mL 0.1% peptone-0.05% thioglycolate were added and the entire contents were homogenized in the original pouch by the Stomacher. Three milliliters were taken and diluted with peptone-thioglycolate. Aliquots were pour plated on Botulinum Assay Medium (BAM) (Huhtanen, 1975) and incubated at 37°C for 2 days in an anaerobic chamber flushed with a N_2-H_2-CO_2 gas mixture. This permits growth of the few indigenous anaerobes that would survive heat shocking as well as the inoculated *C. sporogenes*. Therefore a corresponding uninoculated package was enumerated each time that an inoculated package was. Initial counts in the uninoculated packages were usually less than the minimum detectable level of 10^2 CFU/g. Whenever the counts in the uninoculated packages approached or equaled those in the inoculated, the storage trial was terminated because the identity of the counts would then be in doubt. In some experiments clostridia were inoculated onto ground corned beef, and growth was observed by gas production and swelling of the vacuum pouches. A corresponding set of uninoculated pouches were also observed.

RESULTS & DISCUSSION

Natural flora

Atomic absorption analyses showed that the initial two batches of corned beef contained 1.24 ± 0.02% (mean and standard error of the mean) and 2.14 ± 0.13% NaCl (1.7% and 3.1% brine, respectively). This was less than the desired 1.5% and 2.5%, but the higher salt level was within the probable range of current commercial products. More importantly, there was a 0.90% difference in salt levels between the two samples. Samples were stored at 5°C, 11°C, and 16°C. After 14 days, some of the pouches that had been stored at 5°C were transferred to 20°C or 27°C to simulate a likely pattern of abuse.

Initial counts of facultative microorganisms on the cooked corned beef were approximately 10^4 colony forming units (CFU) per gram (Fig. 1). Growth was highly dependent on temperature. The counts exceeded 10^6 CFU/g after 2 days at 16°C, 4 days at 11°C, and 7 days at 5°C. When the corned beef was placed at 20°C or 27°C the growth was immediately very rapid although probably limited because the counts were already at 10^7 per gram. There was no strong evidence for the salt levels affecting growth. The 1.24% salt corned beef consistently had slightly greater counts through the growth phase, but the difference was never greater than 0.7 log cycle. Similar conclusions were reached by Kraft (1983) who found a 25% reduction in the salt content of hams would be acceptable insofar as microbiological spoilage was concerned. Whiting et al. (1984) reported little effect of salt reductions from 2.4% to 1.6% in frankfurters on the growth of the natural aerobic or anaerobic flora.

Staphylococcus aureus

Another set of pouches containing corned beef from the same batch as above was inoculated with 1×10^4 CFU *S. aureus*/g and stored under the same set of time-temperature conditions (Fig. 1). The initial counts were approximately 3×10^3 CFU/g indicating good survival of the inoculated cells.

Growth was highly temperature dependent, the rate of growth at 16°C was very similar to the indigenous flora. At 11°C, the staphylococci growth rate was much less than the indigenous flora, and at 5°C the staphylococci did not grow. However, after 14 days of no growth at 5°C the staphylococci showed very rapid growth when the temperature was elevated to 20°C or 27°C.

The two salt levels did not appear to affect the growth at any temperature. The 1.24% salt corned beef had somewhat lower staphylococci counts after 11 days at 11°C than did the 2.14% salt corned beef; this may be sampling variation or it may reflect the poor competitive ability of the staphylococci under less favorable conditions (Smith et al., 1983). The slowing of growth on the 1.24% salt corned beef during the second day at 20°C or 27°C may also reflect this competition or the beginning of the stationary growth phase. This inability of staphylococci to compete and grow more rapidly than the normal flora was also observed by Whiting et al. (1984) on reduced-salt frankfurters.

Clostridium sporogenes

Another set of cooked corned beef was prepared which contained 1.6 ± 0.2% and 2.4 ± 0.1% salt (2.4% and 3.6%

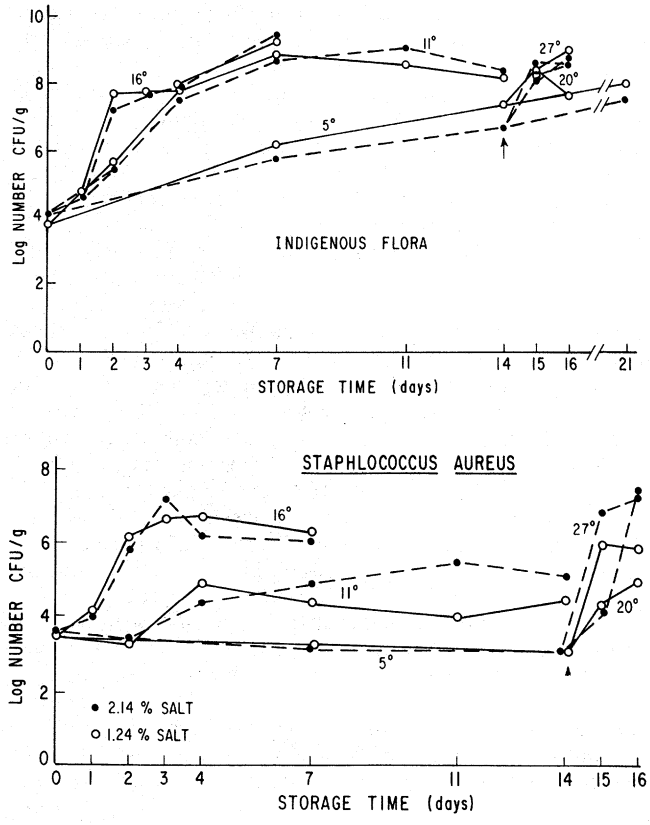

Fig. 1—Growth of indigenous facultative organisms and Staphylococcus aureus in cooked and vacuum packaged corned beef stored at various temperatures. Some packages were stored at 5°C for 14 days, and then shifted (indicated by ↑) to 20°C or 27°C for 1 or 2 days. Values are averages of two runs.

brine, respectively) and had average residual nitrite levels of 57 ± 2 ppm and 39 ± 1 ppm, respectively. Some of these pouches were inoculated with 10^4 spores of *C. sporogenes*/g. All pouches were vacuum packaged, heat shocked, and stored at 16°C, 11°C, and 5°C for up to 14 days. After 9 days, designated pouches stored at 5°C were transferred to 30°C. Corresponding uninoculated samples were also enumerated on BAM media and their counts were always at least 2 log cycles less.

The growth of *C. sporogenes* was slower than the other organisms but the same pattern reappeared (Fig. 2). Growth was moderate at 16°C, slow at 11°C, and inhibited at 5°C. Raising the temperature to 30°C after 9 days at 5°C resulted in an extremely rapid growth. The growth on the 1.6% salt corned beef was slightly greater at 11°C and 16°C.

This growth on corned beef contrasted with the absence of growth in frankfurters (Whiting et al., 1984). A likely explanation is the lower residual nitrite levels in the cured and cooked corned beef when the spores were inoculated, compared to conditions in the frankfurter batter where spores were added soon after emulsifying (Holley, 1981; Hauschild, 1982; Pierson and Smoot, 1982; Robinson et al., 1982).

The relationship between clostridial growth and salt concentration was further examined by experiments in which cooked corned beef containing 1.2 ± 0.1% salt and 72.5 ± 1.2% water was ground and additional salt was added to portions to give a precisely defined series of salt levels. The salt concentration of 1.2%, 1.8%, 2.5%, 3.2%, and 4.0% had calculated brine concentrations of 1.6%, 2.4%, 3.3%, 4.2%, and 5.2%, respectively. Pouches were inoculated with 1.4 X 10^2 or 1.4 X 10^4 spores/g, heat shocked, and stored at 20°C for up to 60 days. A complete set of uninoculated pouches of corned beef were also heat shocked and stored. The appearance of gas and pouch swelling is shown on Fig. 3, the ordinate is the percentage of pouches with definite presence of gas although not necessarily enough for swelling. At 4% salt only a few small gas bubbles were produced that disappeared with continued storage. With the lower inoculation level, gas production was inconsistent at 4.0% salt; no gas production was observed during the second run. No gas appeared in the corresponding uninoculated packages except at a slow rate in the 1.2% salt samples.

The larger inoculation caused a more rapid appearance of gas at all salt levels. This influence of inoculation size has been commented on by Riemann et al. (1972), Holley (1981), and Pierson and Smoot (1982). The inhibitory effect of salt appeared to become a significant factor at and above 3.2% salt, levels above many cured meat products. It is widely known that inhibition of growth and toxin production by proteolytic *C. botulinum* by salt alone requires at least 8.5% salt (Rieman et al., 1972; Pierson and Smoot, 1982).

The influence of nitrite was determined with a similar experiment. From corned beef with 1.3 ± 0.4% salt, lots containing 1.5%, 2.5%, and 3.5% salt were prepared, all with a residual 45 ppm nitrite. Calculated brine concentrations were 2.4%, 3.6%, and 5.4%. To some of the pouches with 1.5% and 2.5% salt, nitrite solutions were added to make a total of 150 ppm sodium nitrite. The pH of these samples averaged 6.04 ± 0.04. Most of the inoculated pouches with 1.5% and 2.5% salt and residual nitrite showed gas after 1 and 2 days at 20°C (Fig. 4). With additional nitrite this was delayed to 10 and 25 days, respectively. The 3.5% salt-45 ppm nitrite showed only trace of gas in a few packages after 35 days at 20°C. Gas production in the corresponding uninoculated pouches appeared much later than in the inoculated pouches in all of the salt-nitrite treatments.

Montville (1983) reported that growth and toxin production by *C. botulinum* can occur in media while gas production may be delayed or absent. Therefore, in the final experiment clostridial growth was also determined. Batches of ground corned beef contained 1.6 ± 0.2% and 2.4 ± 0.1% NaCl with residual 42 ± 1 ppm nitrite (2.4 and 3.6% brine, respectively). Supplemental salt to make 3.5% was added to the latter batch (5.6% brine). Portions of the 1.5% and 2.5% NaCl batches had sodium nitrite added to give 150 ± 10 ppm. Uninoculated controls were also run and a particular treatment was stopped when the counts in the uninoculated pouches equalled those in the inoculated. Gas production and growth were followed during incubation at 20°C.

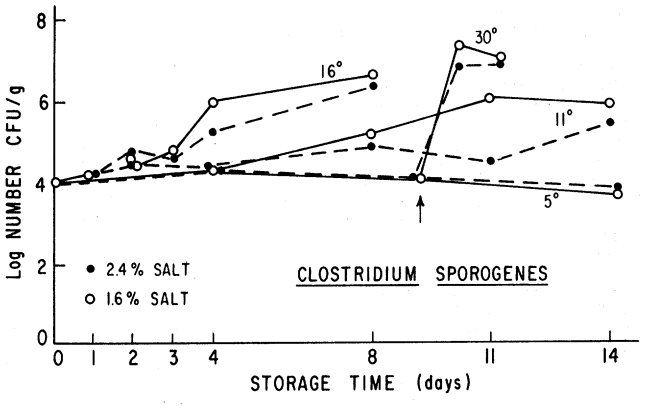

Fig. 2—Growth of anaerobic bacteria in cooked and vacuum packaged corned beef inoculated with 10^4 spores/g Clostridium sporogenes and stored at various temperatures. Some packages were stored at 5°C for 9 days, then stored at 30°C for 1 or 2 days (indicated by ↑). Plotting was stopped when the number of colonies/g in the uninoculated packages equaled the number in inoculated packages. Values are averages of two runs.

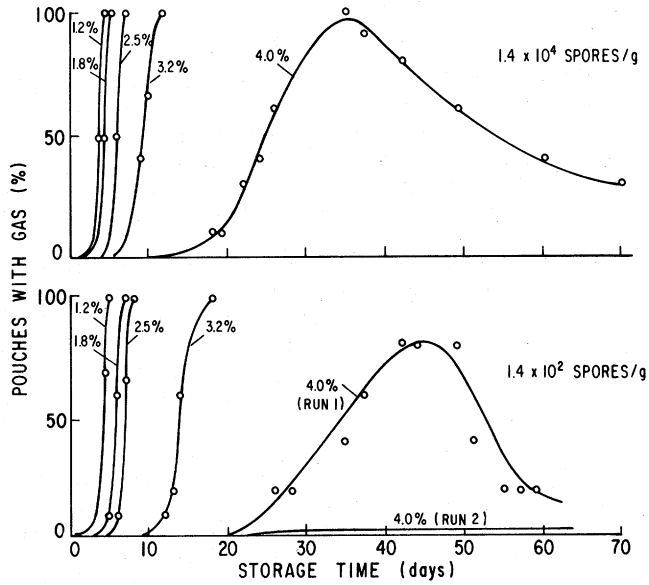

Fig. 3—Presence of gas in vacuum packages of cooked corned beef inoculated with C. sporogenes and stored at 20°C. The corned beef contained from 1.2% to 4.0% salt and was inoculated with either 1.4 X 10^2 or 1.4 X 10^4 spores/g. Values indicate the percentage of packages containing gas and represent two runs of five packages per treatment.

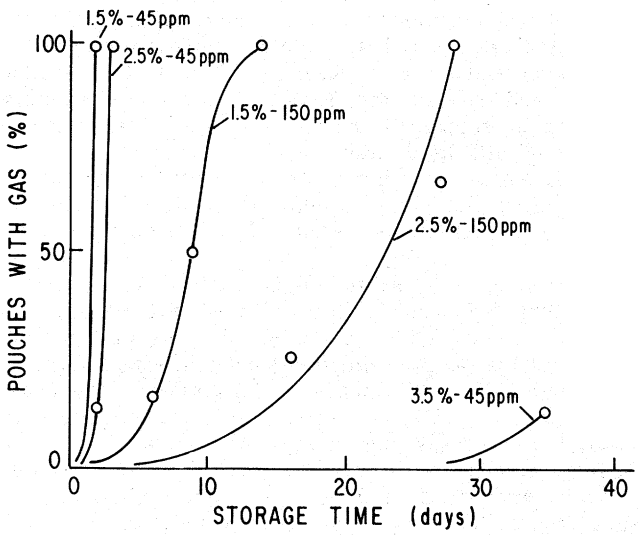

Fig. 4—Presence of gas in vacuum packages of cooked corned beef inoculated with C. sporogenes. The corned beef contained 1.5%, 2.5%, or 3.5% salt and residual nitrite of 45 ppm or added nitrite to 150 ppm. Inoculation was 1.3 X 10^4 spores/g and packages were stored at 20°C. Values indicate the percentage of packages containing gas. Each treatment contained seven packages.

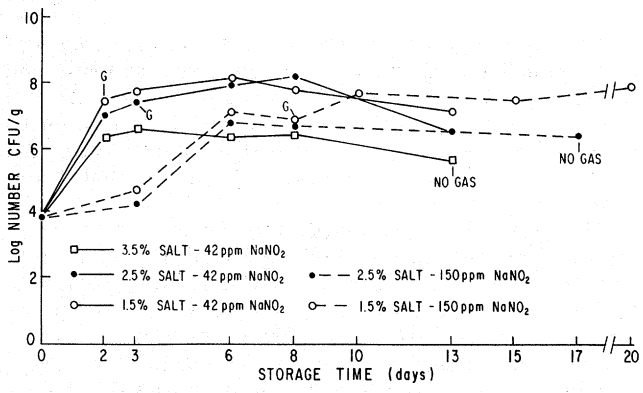

Fig. 5—Growth and gas production in vacuum packages of cooked corned beef inoculated with C. sporogenes. The corned beef contained various salt and nitrite levels. Storage was at 20°C. The time when half of the packages in a treatment contained gas is indicated by "G." Plotting was stopped when the number of CFU/g in the uninoculated packages equaled the number in the inoculated packages.

The three treatments containing only residual nitrite showed a rapid 2 - 3 log cycle increase in cell numbers that was moderately affected by salt level (Fig. 5). Gas appeared quickly in the corned beef containing 1.5% and 2.5% salt but had not appeared in the 3.5% salt samples at 13 days when this treatment was terminated. Corned beef with added nitrite had growth delayed approximately 3 days but cell populations exceeded 10^6 CFU/g after 6 days. The growth curves for 1.5% and 2.5% salt corned beef with 150 ppm nitrite were nearly identical during the growth phase; however, gas production occurred in a majority of the 1.5% salt-150 ppm nitrite sample after 9 days, but never occurred with 2.5% salt-150 ppm nitrite.

CONCLUSIONS

THESE RESULTS SHOWED that the temperature of storage or abuse was more important in determining the growth of indigenous microorganisms, *S. aureus,* and *C. sporogenes* than a reasonable salt reduction from the current industry averages. With refrigeration at 11°C, the normal flora outgrew both staphylococci and clostridia, even though large inocula were used in these studies.

The importance of considering nitrite levels with clostridial growth was apparent; reduction of both salt and nitrite from the current practice would increase the risks of clostridial growth. Clostridial growth was more vigorous when residual nitrite only was present. These results are particularly applicable to contamination occurring after curing and cooking. Further work is in progress on survival and growth of spores during the curing process.

This work confirmed observations that gas production can be a very misleading indicator of clostridial growth. When conditions become less favorable (higher salt and nitrite levels), gas production was inhibited much more than actual growth. This implied that a complete evaluation of the risk of botulism in reduced-salt meat products should not rely on gas production alone, but should include enumeration and toxin assays with *C. botulinum.*

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